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THE METAMORPHOSIS OF FILARIA SANGUINIS HOMINIS IN THE MOSQUITO.

BY

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(*Communicated by* DR. COBBOLD, F.R.S.)

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XIII. *The Metamorphosis of* Filaria sanguinis hominis *in the Mosquito*. *By* PATRICK MANSON, *M.D., Hong Kong*. *Communicated by* DR. COBBOLD, *F.R.S., F.L.S.*

(Plate XXXIX.)

Read March 6th, 1884.

SIX years ago I described the metamorphosis undergone by the embryo *Filaria sanguinis hominis* in the body of the mosquito *. I hoped that (considering the practical importance of a correct knowledge of the life-history of this parasite) the statements I then made would, long ere this time, have been thoroughly confuted or confirmed. If we judge from the wideness of its distribution and the grave character of the diseases it gives rise to, the *Filaria sanguinis hominis* is infinitely the most important of the animal parasites attacking man, much more important than *Trichina* or *Echinococcus*. Biologically the story of its life-history is interesting, and even more wonderful than is that of either of these parasites. Nevertheless, although from both a practical and a scientific point of view it is important to have this assertion of mine about the rôle of the mosquito cleared up, it still remains but half confirmed, half believed.

With the exception of Lewis in India †, Myers in Formosa ‡, and Sonsino in Egypt §, I do not know that any one has worked seriously at the subject. And although both Lewis and Sonsino have confirmed my statements as to the entrance of the *Filaria* into the mosquito, and followed up part of the metamorphosis, neither of them has advanced his observations so far as to be able to confirm my statements as to the later stages of this, or positively to prove that the mosquito is, or is not, the intermediary host.

Some eminent helminthologists in England accept my statements and endorse the inferences I have drawn—Cobbold for example. But in other quarters, so far from securing acceptance of my theory, the work of Lewis, on account of the hesitation and scientific caution with which he expresses himself, has had the effect of inducing a certain amount of scepticism. Leuckart is sceptical; and of course the scepticism of so eminent an authority is of great weight in influencing opinion, especially in Germany. Some of our own zoologists, also, I understand, share the views of Leuckart.

Recently I received a copy of a lecture by Mr. B. Scheube of Leipzig entitled 'Die Filaria Krankheit.' In this very excellent lecture occurs the following passage:—"Der englische Parasitolog Cobbold hat Manson's Ansicht von der Entwickelung der *Filaria sanguinis hominis* adoptirt und auf die Analogie mit der *Filaria medinensis* hingewiesen, welche ihre Metamorphose im Süsswassercyclopen durchmacht. Von Leuckart || dagegen werden gegen die Manson'schen Beobachtungen aus mehreren Gründen starke

* Proc. Linn. Soc. March 7th, 1878. China Customs Medical Reports, Sept. 1877.

† 14th Ann. Rep. of the Sanitary Commissioner with the Government in India.

‡ China Customs Medical Reports, March 1881. 21st issue.

§ Medical Times and Gazette, May 13th, 1883, p. 494; Sept. 22nd, 1883, p. 340.

|| 'Die Parasiten des Menschen,' I. 2. Aufl. p. 85. Leuckart's Jahresbericht für 1875–79, p. 163.

Zweifel erhoben, welche auch die späteren Mittheilungen von Lewis nicht beseitigen konnten. Letzterer constatirte zwar ebenfalls, dass die *Filaria sanguinis hominis* in den Magen der Muskitos übergeht und hier gewisse Veränderungen erleidet; er fand auch bei denselben jugendliche Nematoden, die möglicher Weise einen weiteren Entwickelungszustand der aufgenommenen darstellen, aber es ist durchaus nicht sicher, dass dem in Wirklichkeit so sei. Von einer anderen Seite konnten die Manson'schen Beobachtungen nicht bestätigt werden. Myers wollte dessen Versuche auf Formosa, wo die *Filaria*-Krankheit selbst nicht autochthon vorkommt, nachmachen, kam aber zu dem Resultate, dass die *Filaria*-Embryonen von den Muskitos vollständig verdaut wurden . . . . Nach alledem müssen wir die Frage über die Entwickelung der Filarie noch als eine schwebende betrachten." These words, I presume, represent the attitude of the scientific and professional mind, on the continent at all events, on the subject of *Filaria*-metamorphosis. My assertions may be true, but in all particulars they have not been confirmed, and the evidence I formerly adduced cannot be considered sufficient.

Impressed by the practical importance and scientific interest of this matter, and being naturally anxious that what I had stated and knew to be true should be accepted, and failing decided confirmation from other observers, I thought I could do some service to my profession and this branch of helminthology if I again went over the ground I had twice before trodden. With these and other criticisms before me I have done so, and now endeavour by a short statement of what I found, once for all to settle the matter. This statement I supplemented with a series of illustrative drawings carefully made from fresh preparations and drawn to scale. And, in order that I may quote the testimony of others, I have shown the whole series of my experiments and preparations to Drs. Macleish and M^cDougall, medical men residing in Amoy, and perfectly competent judges on such points. I have their permission to say that they believe my descriptions and drawings to be in the main correct. Further, I have forwarded to friends in England and elsewhere microscopic preparations which, should the frail structures they contain retain the appearances they had when they left my hands, cannot fail to satisfy the most cautious and scientific mind.

There are three ways by which we may settle the relationship of the mosquito to the *Filaria*:—

1st. By tracing the *Filaria* into this insect, witnessing its metamorphosis therein, and finally its escape at an advanced stage of development.

2nd. By showing that it is only in those mosquitoes that have fed on filariated blood that the metamorphosis of a nematode occurs—that no such phenomenon can be witnessed in mosquitoes fed on non-filariated blood.

3rd. By filariating a man, or a lower animal, by means of *Filariæ* metamorphosed in passing through the mosquito.

The first two of these methods I have employed; the sequel contains a description of my results. The third test of my theory remains to be applied. For myself I have not sufficient hardihood or scientific fervour to attempt it in my own person, but I am convinced that a properly planned and conducted experiment would lead to positive results. Both Lewis and Sonsino, besides other observers, confirm me in the statement that the

mosquito, when feeding on the blood of a filarious subject, imbibes the *Filaria.* Lewis confirms me in the statement that nematodes at a stage of development slightly more advanced than those found in the newly ingested blood are also to be found in the mosquito. But Lewis says he could not satisfy himself that these two forms belong to one and the same species of nematode. Neither he nor Sonsino has found advanced forms, so advanced that from their appearance they would be justified in concluding they were fit for independent life. Possibly, suggests Lewis, the more advanced forms are embryos of some other species of nematode, and not the *Filaria sanguinis hominis* at all. In my observations I paid particular attention to this point. If I succeed in connecting the more advanced forms of nematode embryo, as seen by Lewis, with the unquestioned embryo *Filaria sanguinis hominis* found in newly ingested blood, and with the later forms which I had already described as being equipped for independent life, then there is no longer any room for doubt about the rôle of the mosquito, and I can claim that Lewis's observations confirm my own.

I would remind the reader that the parasite called *Filaria sanguinis hominis* is a minute nematode embryo which in certain warm countries is found in the blood of Man. It is the offspring of a mature *Filaria* living in the lymphatic vessels. The young find their way into the blood-circulation along with the lymph.

No form intermediate between the mature *Filaria* and its embryo has hitherto been found in Man. There is no evidence of growth about the embryo, neither has any provision in its structure by which one might suppose it could pass from one man to another been discovered. It seems most probable, therefore, that like many other parasites it requires the services of an intermediary host—1st, to remove it from the body of the host; 2nd, to nurse it till it becomes equipped for independent life; 3rd, to place it in a position for obtaining access to its final host.

In our search for this intermediary host we must be sure the animal we encounter can fulfil these conditions. In addition to this there are two other circumstances in connexion with the *Filaria* which must also be recognized in fitting it with an intermediary host. Such an animal must have a geographical distribution corresponding to that of the *Filaria*; and it must also be nocturnal in its habits. The reason for the first of these conditions is self-evident; the latter is indicated by the curious fact in the history of the *Filaria,* that the embryo appears in the blood only at night. This peculiarity in the habits of the *Filaria* has now been so frequently demonstrated and confirmed that I need concern myself here only with the mention of it.

There are many reasons why one should regard the mosquito as the animal most likely to fulfil all the conditions required. These reasons I will not enlarge on. I will confine myself to the description of what actually occurs in the relationship of the mosquito to the *Filaria,* feeling satisfied that in this is the best proof that the mosquito is the true intermediary host of the *Filaria sanguinis hominis.*

In experimenting with the mosquito there are two points I was careful in attending to; I employed the proper species of insect, and I took care that its only food was the blood of a filariated man. Those who would repeat my experiments must bear these two points in mind. The plan of procedure I found most convenient was as follows:—I

engaged a man in whose blood the *Filaria* abounded. A large square wooden frame (10 ft. $\times$ 10 ft. $\times$ $6\frac{1}{2}$ ft.) covered with mosquito-netting and provided with a door was erected in a room where mosquitoes abounded. Under this mosquito-house the man slept, the door of the house being closed some hours after he had gone to bed. Next morning the mosquitoes that had entered by the door, and preyed on the man's blood, were found clinging to the inside of the netting, their abdomens distended with blood. These my servant captured under a wine-glass, and after paralyzing them with a whiff of tobacco smoke transferred them each to a dry phial covered with gauze. When the insects had recovered from the effect of the tobacco and ascended the side of the bottle for a little distance, a small quantity of water was introduced through a pipette. Latterly I employed large-mouthed stoppered bottles, holding about two ounces, instead of the phials. Tobacco could in this way be dispensed with, and a considerable mortality avoided. The bottles were dated and put under a dark shade. When the weather became cool I got better results by placing the bottles in an incubator, where the insects were kept in a damp atmosphere at a temperature ranging from 80° to 85° Fahr. In this way I endeavoured to imitate nature. Every morning during many weeks a fresh crop of *Filaria*-charged mosquitoes was gathered. For the purposes of this investigation quite a thousand insects had to be provided. From time to time, as required, they were removed from the bottles after being killed by chloroform. The legs and wings and head were torn off. The thorax was then separated from the abdomen, each being placed on a separate slide. In the case of the abdomen the contents were expressed by rolling a penholder from the free to the severed thoracic end; a cover-glass was then placed on the expressed blood. If necessary, a little water or sulphate of soda solution (sp. gr. 1050) was used to soften the blood and allow of the easy removal of the two large ovisacs, which, when crushed, obscure the slide very much. The thorax is best treated by being broken up, and teased out with a couple of sharp needles in a droplet of water, before the cover-glass is applied. Thus prepared, an inch objective readily detects the parasites among the tissues of the insect. To preserve the *Filariæ* thus displayed the cover-glass must be carefully lifted up, and what remains on the slide allowed to dry, the large pieces of mosquito débris being first picked off with a fine forceps. After drying for a day or, better, two, it may be stained with an aniline dye (gentian violet answers very well), and after washing and redrying mounted in Canada balsam. In the case of *Filaria* in the blood from the abdomen of the mosquito it is only necessary to dry the preparation before mounting in balsam. The red colour of the blood gives sufficient contrast to display, at all events, the outline of the parasites. Whenever practicable, fresh and unstained preparations should be examined; for while stained specimens show the outline well enough, they are useless in studying the details of internal structure.

My mosquito-house and filarious patient were visited by three species of mosquito, perhaps four. All of these were capable of imbibing the *Filaria*, but only one of them is, I believe, capable of conducting the metamorphosis to a successful conclusion. Both of the impotent species are of the kind known as tiger mosquitoes, *i. e.* their bodies and legs are banded, or flecked with white. One is quite a large insect, half an inch in length, with numerous sooty, easily detached scales; the eggs of this species when

deposited on the surface of water, do not keep together in a boat-shaped cluster, as do those of the true *Filaria* mosquito, but float about separately, tending to adhere to the sides of the bottle. There is no danger of confounding this species with the true *Filaria* mosquito. The other species of tiger mosquito is smaller, and in this way is liable to be mistaken for the *Filaria* mosquito; but a close inspection shows it to be smaller in size and darker in colour than the true species; and, in addition to these points of distinction, the little flecks of white and white bands on legs and body serve, with a little care, to prevent mistake. I have often found the *Filaria* in the blood in the abdomen of both of these mosquitoes, but never in such numbers as one finds them in the true *Filaria* mosquito. In the thorax I have found them slightly advanced in development in the larger tiger mosquito; but although I have examined a considerable number of this species on the third day after they had fed, I have not found the *Filaria* at this later period, when in the true *Filaria* mosquito they abound, and cannot readily be overlooked. I would not be positive on this point, as my observations on the tiger mosquito are not sufficiently numerous; but my impression is that both species are incapable of acting as efficient intermediary hosts.

The true, or what I believe to be the true, *Filaria* mosquito is a brown insect, about $\frac{3}{16}$ of an inch in length, snuff-brown in colour, without any particular markings whatever. In some specimens, and especially some days after feeding, and when her eggs have been deposited, there is an appearance of banding about the abdomen; but there are no distinct markings, as in the case of the tiger mosquitoes. It appears to thrive best when the thermometer ranges from about 75–84° Fahr. In the very hot weather it disappears, or is languid, and the rate of maturation of the ova (in those specimens one can procure) is too quick apparently for the metamorphosis of the *Filaria*. Consequently, experiments in June, July, and August may not be uniformly successful. In Amoy, September and October are the best months for observations on this species. The female alone is the blood-sucker. She feeds but once if not disturbed, and lays her egg about the fourth or fifth day afterwards. Some lay on the third day, and some do not part with their ova till the seventh. As a rule, they die soon after laying, but a few survive till the sixth or seventh day, and in these alone is the metamorphosis of the *Filaria* completed. Perhaps one mosquito in ten attains this age, at all events in confinement. Possibly in normal surroundings a larger proportion survive.

On commencing the study of the metamorphosis of the *Filaria* in the mosquito the first circumstance that strikes one is the largeness of the number of *Filariæ* ingested by the insect. In a corresponding quantity of blood, drawn in the usual way from the finger, not one fourth part of the number contained in the blood of the insect's abdomen would be found. It is quite a common thing to find 30 or 40 *Filariæ* in the minute speck of blood we express from the abdomen. Often this number is exceeded, and more than once I have found them literally in hundreds. This indicates that the mosquito in some way or another is adapted for fishing the *Filaria* out of the blood stream. The proboscis of the insect, as it lies in the lumen of the vessel from which blood is being imbibed, must tend to arrest the parasites as they are swept against it by the stream, just as a stake in a stream of water accumulates straws and sticks; and the lashing

movements of the little animals tend to entangle them still further, and bring them under the influence of the suction-force exercised by the insect.

Arrived in the abdomen of the mosquito the *Filaria* for a short time retains the appearances and movements that it exhibited while in its human host, and which have frequently been described (fig. 1). Presently a delicate, closely set, transverse striation, as if from general longitudinal shrinking, gradually becomes very evident. The sheath also in many cases can be more readily seen, and oral pouting is very distinct. Within an hour of the time of ingestion, the *Filaria* usually casts its sheath, which, by careful searching, may be seen either trailing after it, or lying across it, or somewhere near. After the casting of the sheath transverse striation and oral pouting become still more distinct, and one can almost fancy the parasite has a true mouth surrounded by lips. It is impossible to determine this, however.

Until the casting of the sheath, the peculiar lashing movements of the animal continue; but when this has been effected a change in the character of these movements is in many instances observable. Hitherto they have been free, lashing, purposeless apparently; and although the little animal has kept in perpetual and vigorous motion, it has never changed, materially, the spot it moved in. There has been no locomotion. But when the sheath has been got rid of, the nature of the motion changes to a snake-like wriggle of regular undulations, which cause a definite forward movement (fig. 2). In many specimens this is very striking. If motion is prevented by some insuperable obstacle in one direction, the *Filaria* retreats and tries in another. It is evidently endeavouring to change its locality.

The object of this singular change in the character of the movements exhibited by the *Filaria* is explained if we examine the thorax in a batch of insects at short intervals after they have fed. We tease it up with needles in a little water, or sulphate-of-soda solution, tearing up the muscles of the wings thoroughly, before applying the cover glass. Examined, thus prepared, within a short time, say half an hour, of feeding, all the *Filariæ* are to be found in the abdomen, none in the thorax; after an hour, two or three may be found moving among the muscles of the thorax; after another hour, many more may be detected here, till at the end of 12 or 18 hours, the thorax is found to be full of parasites. Therefore, the movement of progression we see in the *Filaria* that has cast its sheath in the blood in the abdomen, has for its object the migration of the parasite.

Not all, but a very large proportion of the injected *Filariæ* do thus migrate. My former observations were made entirely on *Filariæ* found in the abdomen, or believed to be in the abdomen. Not suspecting this migration, and finding metamorphosis going on in the abdomen, I may have, unwittingly, included some of the viscera of the former in my examinations of the latter. On that occasion I traced out the metamorphosis to its conclusion, and entirely in what I thought, at the time, were abdominal tissues. It is likely, therefore, that migration to the thorax is not a necessary step indispensable for the welfare of the parasite. But it is certainly the usual first step for the animal to take, and it is a fortunate one in the interests of the observer, as in tracing the subsequent steps of the metamorphosis, the ova, which in the abdomen are so annoying from their obscuring the field when ruptured, are not encountered. Lewis was

the first to mention this migration; until I had read his description of his experiments on *Filaria* metamorphosis, I entirely overlooked this significant point.

Most of those *Filariæ* that do not migrate, gradually become granular, their outlines become dim and undefined, and their movements cease. I suppose they are finally digested.

If we examine carefully a *Filaria* that has just reached the thorax, we find that the striation observed in specimens from the abdomen has disappeared; the body is beautifully transparent; there is no sheath or sign of double outline; oral movement still persists, but the general body-movement has slowed down till, in some instances, the animal is almost passive. The body is somewhat thicker and, it may be, shorter, and an obscure undefined cloudiness can be seen in the interior (figs. 3 & 40). How far this dissipation of the transverse striation, slowing of movement, and swelling may be owing to endosmosis of the water that we necessarily employ in mounting the specimen, it is difficult to say. I know that if the young *Filaria* is soused in a large quantity of water, it becomes so transparent that, under a low power, it is readily overlooked or even cannot be seen. Sudden endosmosis, I believe, often ruptures and dissipates it. I therefore, in examining the thorax of the mosquito for the *Filaria* at any time during the first two or three days of the metamorphosis, employed a solution of sulphate of soda (sp. gr. 1050) to tease up the tissues in. After application of the cover glass, the soda solution was gradually diluted by placing a drop of pure water at one edge of the slide, while the solution was withdrawn by a piece of filter paper placed at the opposite edge. The soda solution shrivels, and renders irregular the outline of the *Filaria*, but by gradual dilution, as described, the little animal is rendered plump and natural-looking again. I believe it is the *sudden* immersion in water that is dangerous to the integrity of the *Filaria*. For convenience in description, I divide the metamorphosis into six stages *. What I have now related constitutes the first stage, viz.—ingestion by the mosquito, transverse striation, casting of the sheath, and subsequent migration. At the conclusion of this stage it is quite a common thing to find, with very little searching, 30 or 40 *Filariæ* in the thorax, and very many more can usually be detected half concealed by careful focussing among the viscera and fragments of muscle.

The first noticeable change in the *Filaria* after migration is a shortening and general thickening of the entire body, the extreme tip of the tail being alone exempted from this process. This part retains its original dimensions for a time, while the remainder of the animal continues to swell. Thus the caudal appendage, characteristic of the *Filaria* during the greater part of its stay in the mosquito, is formed. In some specimens a thick hyaline-looking substance seems to cover as an integument the body of the animal from the mouth downwards, stopping short, however, almost abruptly at the root of the tail. The tail in all cases is evidently of the same structure as the interior and mass of the body. In others, the integument I mention is not observable, the tail seemingly

* The reader must bear in mind that this division of the metamorphosis is entirely artificial. No such thing exists in nature. What I describe as stages, in reality overlap each other; the graduations of development insensibly merge one into another.

being differentiated from the rest of the animal simply by its not having partaken in the general swelling (figs. 4–10–38, 41, and 42).

At this stage movement still persists; but as the body swells, motion becomes fainter and more intermitting, till, in many examples, it finally ceases soon after migration has been effected. There is a tendency now for the oral end to become tapered, and more conical, one or more black points being visible at the very end.

The body continues to swell till it has more than trebled its original diameter, and become shorter by one third. Thus the tail comes to look a mere appendage stuck on one end of a sausage-shaped mass.

At first, and often all through the subsequent steps of the metamorphosis, the tail does not correspond with the axial line of the body, but is quite to one side. In others, especially at a later stage, the position of the tail appears to be, and possibly is, axial, although this effect is produced in many instances by the position the animal is viewed in, according as the tail is to right or left, above or below; in both the latter positions it must appear axial, although it may really be quite to one side.

When the body has attained a thickness of about the fifteen hundredth or the two thousandth of an inch it lies sometimes outstretched, sometimes gently bent, occasionally curved on itself, so that mouth and tail are almost touching (fig. 42). The oral end gives the impression that there is an orifice there, but that it is firmly pursed up. Movement of the body is now rarely seen. Occasionally in some there is a slow general bending and extension, and in others a peculiar shivering movement. The tail still at times is vigorously flexed and extended. No organ or structure of any definite kind can be made out even with a high power; only a little in advance of the tail, where the anus is afterwards formed, sometimes an aggregation of minute cells with relatively large nuclei can be seen (fig. 4). The rest of the body is made up of a cloudy granular material enclosed in a very delicate integument. The latter can be made out only after crushing of the body and partial escape of contents, or when the slide has partially dried. In such circumstances we may sometimes see a funnel-shaped mouth into which the delicate integument is reflected (fig. 10).

The thickening of the body, differentiation of the tail, and first indications of a distinct mouth constitute the second stage of the metamorphosis, and usually require two or three days for their completion.

About the third or fourth day we often find the body of the sausage-shaped *Filaria*, which hitherto has shown little or no structure, filled with a crowd of minute shining granules (fig. 11). These, I believe, are nuclei which from extreme transparency and refracting properties, similar to that of the stroma of the animal's body, could not at an earlier stage of development be recognized. In others again, apparently somewhat more advanced, cells to which these nuclei belong become obscurely visible (figs. 12 to 20). These cells are exceedingly minute. Each possesses a distinct nucleus, and together they make up the entire mass of the body and tail inside the delicate cuticle.

About the time the cells become visible the rudiment of the future anus shows itself as a sort of pit or vacuole a short distance in front of the tail (figs. 12, 13, 15, 39, etc.). When viewed in profile this pit or vacuole seems infundibuliform, and the outline of

the margin of the body of the animal is broken at this point. Thus it would seem that here there is a hole in the cuticle. When looked at directly from behind, or in front, it appears round. From its first appearance, and until the last step of the metamorphosis is entered on, granular matter and minute nucleated cells are seen to escape from this point. Very little pressure and sometimes even the mere immersion in water is sufficient to bring about this granulo-cellular discharge from the anus. I do not consider that this discharge comes from inside an alimentary canal, but look upon it as perianal tissue which the exterior tenuity of the walls of the rectum, and the absence at this point of integument, permit to escape, rupture being favoured or brought about by endosmosis of water used in mounting

Coincident with the appearance of anus and cells the mouth advances in development. From being pursed up it seems to open, and gradually four large fleshy lips are fashioned (figs. 16 to 30).

Then a line, at first very faint and broken, shows itself. Running in the axis of the body for the most part, its destiny appears to be to connect mouth and anus. Around this line as it becomes thicker and longer, cells are seen to be arranged (figs. 14 to 25). The line does not seem to extend in all cases quite to the anus, but terminates, as far as one can make out, in advance of this some little distance among certain comparatively large and prominent cells. By degrees this line, the rudiment of the alimentary canal, becomes thicker, and the arrangement of nucleated cells around it forming the walls of the alimentary tube becomes very distinct. In some instances the line itself is manifestly double-tubular. Probably a fine membrane lines it, continuous at the mouth with the skin, and is the lining-membrane of the future alimentary canal.

How this alimentary line is produced it is difficult to say. It is certainly not a process derived from the integument dipping in at mouth or anus; for, in many instances in which it is visible, it is only so about the centre of the animal, and is not traceable into either mouth or anus. My impression is, that this line is produced something after this fashion :—After the cells I have described as filling the body at the beginning of the third stage have been formed, they arrange themselves into two sets. One set goes to the periphery of the body to form future muscular and fibrous walls ; the other set accumulates in single file in the centre of the body in a line extending from mouth to tail. The cells thus arranged in single file divide and subdivide longitudinally as regards the axis of the *Filaria*, the lines of division radiating from the centre of the original file of cells, like the segments of an orange. This would have the effect of making the point of convergence of the lines of cleavage very distinct—the alimentary line. After a time the cells swell out or increase laterally, thus opening out their line of convergence, which thus is converted into a tube, the alimentary canal. This process extends no further back than the anus ; but the central line of cells can sometimes be traced as far back as the tail, and it is principally from having seen this post-anal line of axial cells that I infer this method of explaining the formation of the alimentary line and canal.

While the alimentary line and canal are being formed, cells range themselves, as I have just said, around the periphery of the *Filaria*, lining the skin. But between this

tegumentary set of cells and those forming the alimentary canal there is a distinct interval, a sort of peritoneal cavity, in which at a later stage the alimentary tube moves freely, not being attached at any point save at the anus and mouth (fig. 25).

At the conclusion of this, the third stage of the metamorphosis, the *Filaria* measures from $\frac{1}{100}''$ to $\frac{1}{80}''$ in length, by $\frac{1}{850}''$ to $\frac{1}{500}''$ in breadth, or thereabouts. There is considerable diversity both in size and shape. The mouth is wide open; the tail is large and sickle-shaped, and the cells of the body usually dip into it. The alimentary line runs from mouth to anus; and the cellular nature of the entire animal, with the exception of the integument, is easily demonstrated. Motion is entirely suspended.

Growth hitherto has been very slow, but now, when the fourth stage commences, it becomes rapid, the animal quickly attaining a length of from a seventieth to a fiftieth of an inch. The walls of the alimentary canal can be distinctly traced as the walls of a tube from the open mouth to near the anus, but not quite into structural continuity with the latter. The cellular structure of the mass of the body is often beautifully distinct (figs. 23, 24, 25, 26). The body retreats from the tail, which becomes a mere empty integumental appendage, so transparent that it can with difficulty, in many cases, be made out (fig. 34, 35). The addition of water to the preparation causes the little animal often to rupture after a few minutes at one or two points, a cloud of cells and granules escaping.

In not a few instances a vacuole, similar to that which preceded the formation of the anus, is seen at this stage at a point some little distance behind the mouth. This may be the result of endosmosis, but possibly it is the forerunner of vulva and organs of generation. In one instance (fig. 23) I detected a line among the cells which, from its position, forcibly suggested that it was the rudiment of organs of generation; but as this was a solitary observation, I attach no great weight to it. The vacuole, in the position I mention, is quite a common occurrence.

When the body has attained its maximum thickness the fifth stage commences. The cells, especially in the anterior part, gradually lose their distinctness, and the mouth inclines to purse up, while the animal, as a whole, elongates and attenuates. In some the lengthening and thinning begin at the head and extend backwards, so that if we find such a *Filaria* in the middle of this extension-process, it has a narrow anterior half rather abruptly swelling out into a thick posterior half with the dimensions of the previous stage (fig. 27). Such a specimen resembles in shape a hock-bottle. A very few elongate anteriorly and posteriorly simultaneously; and we may find in one undergoing this process a small segment of the middle of the body still thick and unextended (fig. 28). More generally, however, the extension-process occurs simultaneously throughout the body, the fore part being always rather in advance of the rest (figs. 29, 30).

Sometimes at this stage, just before the mouth closes up, the alimentary tube is seen very distinctly. It moves freely in the body-cavity, accommodating itself to the gentle movements which the anterior half of the *Filaria* now exhibits. The pharynx in such a specimen is distinctly indicated by two short parallel or curved dark lines, terminating at one end in the mouth, at the other leading into the œsophagus (fig. 44). At its other extremity the œsophagus ends in a thick bulbous valvular-like arrangement opening into the intestine, which in its turn may be traced as a distinct tube almost to the anus

(figs. 26, 29, 30). When the mouth closes, as it does presently, all or nearly all t ace of viscera and all trace of cells vanish. The body assumes a fibrous and very transparent look, and little structure can be made out.

The movements just alluded to begin in the neck of the *Filaria.* At first they are of a gentle to-and-fro swaying character; but gradually, as the whole body becomes attenuated, they extend backwards *pari passu* with the attenuation, and until the entire length of the animal becomes more or less animated. These movements do not last longer than a few minutes, at this stage, after immersion in water, differing in this respect very much from what happens at the next stage.

The fate of the sickle-shaped caudal appendage, which up to this time has been so characteristic of the growing *Filaria*, is a little doubtful. It may be that the extreme end of the now truncated body gradually forces its way into the remains of the tail, from which it had before retreated, stretching it so as to have it as a closely fitting integument. More probably the sickle is cast off in a general ecdysis, which about this stage, I believe, occurs. Fig. 46 tends to show that this is probably what really happens; a new skin is shown covering the papillated tail, inside of and quite distinct from that constituting the "sickle." In the example from which this sketch was made, on tracing the skin, continuous with the "sickle," forwards, a point, about abreast of the anus, was reached where it seemed to be peeling off or breaking down.

About this time at the very extremity of the truncated tail, two or three cells become prominent, their nuclei enlarge, and the surrounding protoplasm is increased in bulk. These cells after a time protrude from the general surface, and gradually become converted into the circle of three or four papillæ which characterizes the *Filaria* at the end of this, and during the last stage of metamorphosis (figs. 30, 31, 43, 46, 32, 33). I am not sure if one or two of these papillæ be not the remains of the stump of the original tail; nor am I quite sure of their number, as it is usually impossible to obtain a view in which all can be seen at once. After a time the papillæ spread out like the petals of a flower, extending considerably beyond the margin of the circumference of the body, so that in time they become the broadest part of the animal.

The purpose of these caudal papillæ is difficult to divine. Possibly they are of use in aiding the animal in its future journey through a human host. One can understand how by opening out on any retrograde movement they will prevent this, give a firm foot-hold, so to speak, to the boring *Filaria*, and favour forward movement. They are not oral, as I at one time supposed.

Together, the papillæ, the anus, and alimentary line are very deceptive, and give the idea that this part is really the head of the animal, the papillæ apparently surrounding a mouth with organs of generation opening in close proximity, as is so commonly the case in the *Filariæ*. But I have satisfied myself that in these features we have really to do with the tail of the animal, not the head.

During the progress of these changes the *Filaria* has gradually stretched from perhaps the fortieth to the sixteenth of an inch in length; at the same time its breadth has decreased one half. The anterior end tapers gradually and is then abruptly rounded off, hardly any structure being visible about it. The posterior end also tapers slightly from

the anus backwards, and its very extremity is covered with the three or four papilla just described. Faint indications of an alimentary canal are at times discernible, but further than this no trace of internal structure can be clearly made out. The parasite has now arrived at the sixth and last stage of its metamorphosis, and its stay in the mosquito is about concluded.

Such specimens as I have been describing as belonging to the fifth stage are to be found only about the end of the sixth, and beginning and middle of the seventh day, from 130 to 156 hours after ingestion. The *Filaria* of the sixth stage I have only seen in mosquitoes from 156 to 160 hours after capture, probably about seven days after feeding. Most mosquitoes lay their eggs and die during the fifth or sixth day after feeding, consequently it is a rare thing to find an insect of the age requisite for the maturation of the *Filaria*. Probably of twenty mosquitoes one or two may attain this. I have seen many *Filariæ* in the fourth and fifth stages, but very few in the sixth and last.

During this stage the swaying movements that had commenced during the fifth gradually become more active. The animal is now no longer burst or killed by immersion in water. On the contrary, even as we look at it, the water we have just immersed it in seems to have a vivifying effect on the parasite; its tissues appear to become more consolidated, its motions more active, it seems to feel that it has got into its proper element. It rapidly acquires great muscular power, it wriggles, twists, bends, extends, and lashes about in all directions (figs. 23, 33). As seen with an inch objective it resembles nothing so much as the embryo *Filaria* in the blood looked at through a quarter-inch; the tail, of course, is different, but the character of the movements is exactly the same. One peculiar motion is strikingly like one that is often seen in the blood-*Filaria*. Often it pauses in the midst of its contortions, and suddenly extends itself, remaining outstretched for a moment, its body quivering in a sort of tetanic spasm. Those who have watched the movements of the *Filaria* in the blood will recognize what I mean. In other examples the wriggling movement is not seen, the activity of the animal being expended in frantic rushes forwards and backwards, and in every direction. I presume this description of movement belongs to a slightly later stage than does the wriggling motion; probably it is the motion suited to the time when the mosquito has died and fallen into water, and the parasite has obtained its liberty.

Frequently, during the contortions of the animal, it turns its mouth towards the observer, and seems to be endeavouring to uplift the cover-glass. During this movement we can see that the mouth is pursed up into a cone, the lips being firmly approximated; and, around the spot where the mouth must be, a number of exceedingly minute horny-like papillæ are arranged. This may be the boring-apparatus. But the motions of any animal I have examined at this stage have been too incessant to permit a satisfactory view, and these boring-papillæ may be, to some extent, matters of imagination. So incessant are the movements that it is quite impossible to obtain satisfactory measurements. After watching one for over an hour, and despairing at length of getting it to slow down, I killed it by instilling a little osmic-acid solution below the cover-glass. It then gradually slowed down, stretched out, and died. I found it to measure $\frac{1}{16}'' \times \frac{1}{850}''$. In a good light this particular parasite was perfectly visible to the naked eye.

I believe the *Filaria*, after it has attained this final stage, has but a very short time longer to pass in the mosquito. I am inclined to think it preys on the tissues of the insect, and thereby contributes to its death. For, in examining mosquitoes found dead on the surface of the water, and which I know could have died only a very short time before, I have been struck with a singular absence of viscera and muscular tissue—the thorax seemed but a hollow shell. Again, some mosquitoes, even after death, cling or stick to the sides of the vessel containing them; in such specimens, also, I have remarked an absence of viscera and muscular tissue. My impression is, that these have been consumed by the *Filaria*, that the death of the insect had been thereby hurried, and that when this occurred the parasite bored its way out of the body, and thence sped to the water. I have never found a *Filaria* in the last stage of the metamorphosis in a dead mosquito; often, however, I have found them at all stages but this, even up to the time when they measure the fortieth or the thirtieth of an inch in length. The final stretching process must be rapidly effected, lasting probably an hour or two; and, as during it the *Filaria* becomes more than double in size, there must be expenditure of the tissues of the intermediary host to provide pabulum for this rapid growth. Hence, in some measure, it comes about that one rarely catches the *Filaria* in the last stage of growth, and that the dead mosquito has an empty thorax.

Such, I believe, is a true description of the life of *Filaria sanguinis hominis* in the mosquito. Some of my interpretations may be wrong, but all the principal facts I have stated are true. I have verified them over and over again.

I can understand how, if fig. 1 and fig. 34 are compared, some doubts may arise as to their connexion. The contrast between fig. 1 and fig. 23, or between fig. 23 and fig. 33, is great—so great that unless one had carefully traced the connecting links step by step, one would positively declare them organisms of entirely different species. Over and over again, when working at the *Filaria* of the fourth or fifth day, I have hesitated to believe it was really the outcome of the *Filaria* I had seen in the abdomen of the mosquito an hour or two after feeding; and I have turned to mosquitoes of the first, second, and third days to reassure myself of the migration to the thorax, the differentiation of the tail, the swelling of the body, the formation of the mouth, anus, and alimentary lines before I was at my ease as to the genesis of the particular organism that I was specially engaged in studying. But now, to my mind, there does not exist the slightest doubt in the matter.

Besides being able to trace the gradation in mosquitoes of different ages, we often encounter specimens of the *Filaria*, at all stages of development from fig. 3 to fig. 14, in one and the same mosquito. Sometimes in an insect in which a *Filaria* like fig. 14 is found, many others, at all stages of development, from fig. 14 to fig. 24, may be encountered. And so with the latest stages: fig. 23 may be found in a slide in which fig. 33 is moving. This gradation is very striking, and clearly indicates the connection between the *Filaria* ingested by the mosquito and the *Filaria* about to leave it.

The most difficult step to follow, and the one over which Lewis apparently hesitated, is that represented from figs. 4–12. If, however, insects of from twenty-four to forty-eight hours after feeding are studied, transition-forms are found in abundance. Figs. 40,

41, 42 are from the same insect. I have seen and sketched specimens resembling figs. 3, 8, and 12 in the same field of the microscope, and lying across each other.

In the little caudal appendage that characterizes the *Filaria* from the second to the fifth stage we have a strong argument for believing we are concerned with the same species during all these stages. It ceases, however, to be distinctive towards the end of the fifth and during the sixth stage; but here a curious circumstance comes to our assistance in establishing identity. The thick cellular body of the fourth and commencing fifth stages is motionless; but as the anterior part fines down and stretches out, it at the same time acquires movement. A difference also obtains between these two parts as regards their capacity for being stained with gentian violet. The posterior and cellular part takes the stain with difficulty, or not at all; whereas the anterior fibrous and active part takes the stain rapidly and deeply. Now we sometimes find a *Filaria* with a long stretch of the fore part of the body in thickness and general appearance like that of fig. 31, and in very active movement, dragging after it a limp and seemingly paralysed posterior and cellular part, rupturing readily on immersion in water, and showing all the characteristics of the cellular stage. Further, on applying the stain to such a specimen, the active fore part is deeply affected, whereas the limp hind part is but slightly tinged*. Such a specimen bridges over the step from figs. 23 to 33. Catching specimens such as fig. 27 and fig. 28 in the act, so to speak, of stretching, distinctly establishes that the *Filaria* of the sixth stage is but an advanced form of the *Filaria* of the fourth, and, by a similar process of reasoning, of the first, and of the *Filaria* in the human blood.

I can imagine that some may found an objection to the theory of the action of the mosquito on the fact that, of the hundreds of *Filariæ* entering a certain mosquito, only a dozen or so will attain the more advanced stages of development; and that, of those that do thus advance in development, most perish in consequence of the death of the insect before the *Filaria* is equipped for independent life. It is true that the great majority cease to develop at a very early stage; others, after advancing a little, seem to die and wither. Fig. 36 is from such a specimen. Perhaps only certain tissues or organs of the mosquito favour complete development, and in their blind wandering few of the *Filariæ* reach these. But these facts are no argument against the theory that the mosquito is the intermediary host of the *Filaria*: indeed, on the contrary, they are in entire consonance with what we know of the life-histories of other parasites. Of the millions that leave the parent, but one or two ever reach maturity. The journey from the uterus to the spot where maturity is attained is beset with dangers, the measure of these dangers being the prodigious numbers that start on the journey.

* Strange to say, the fully developed *Filaria*, as that represented in figs. 32, 33, does not take the stain well. It seems to me that the age of the integument has something to do with the facility with which the stain is absorbed. Specimens, as at figs. 22, 23, 25, do not stain well, I believe the reason being that the integument in these is old and, presumably, dense. But the anterior half of fig. 27 would stain well; it is the growing half, and, I think, has just undergone ecdysis, and the new skin is easily permeated by the dye. Its posterior half, however, will not stain at all, or but very slightly and unevenly; the old skin is not yet cast, and is dense and impervious. The new skin, after a time, as it ages, also becomes dense, so that by the time the animal has grown to the size of the original of fig. 33, it is impervious, and will hardly allow the passage of the gentian violet.

Frequently during my study of the metamorphosis in the mosquito, it has occurred to me that the later stages of what I describe as the *Filaria* of man in the mosquito, may really be stages in the development of a purely insect parasite like *Gordius* or *Mermis*, and not the *Filaria sanguinis hominis* at all. Perhaps, I conjectured, this animal may escape from the body of the mosquito into water, where it may acquire organs of generation and breed, and its young may afterwards enter the body of the mosquito larvæ, and so complete the cycle. But if this is the case, there must be a time in the histories of the human *Filaria* and this hypothetical animal when it is impossible to discriminate between them. I have never been able to find a break in the chain of development.

Moreover, if the mosquito is liable to be infested by a nematode parasite peculiarly its own, and if the developmental forms I have been describing belong to such a parasite, then we ought to find them in mosquitoes that had fed on non-filariated, as frequently as we find them in mosquitoes fed on filariated blood. If one filarial form could be found in a mosquito fed only on non-filariated blood then my theory about the rôle of this insect is in danger of falling to the ground.

This is a point I gave some attention to. Simultaneously with observations on mosquitoes fed on the blood of my filarious subject, I carried on others on mosquitoes caught in the mosquito-nets of coolies and servants living in my house. The result was very striking. I carelessly assumed that my servants were free from *Filaria*, and did not examine their blood, but trusted to the mosquito to pick out for me those who might be filarious.

Two mosquitoes were brought to me by coolie No. 1. One of these I examined at once—no *Filaria*; the second I kept till the fourth day before examining it—no *Filaria*.

Coolie No. 2 brought two mosquitoes. I examined one on the first day but found no *Filaria* in the blood in the abdomen. The second I kept till the third day. I examined its thorax very carefully, and then had the mortification of finding a sausage-shaped body exactly like that represented at fig. 12. There could be no doubt about its nature. I thought my theory was upset, and all my work useless. At 9 P.M., however, I examined blood drawn from this man's finger, and had the satisfaction of finding in it plenty of *Filariæ*; subsequently he contributed *Filaria*-charged mosquitoes for my investigation of the metamorphosis. The mosquitoes he brought me were often devoid of *Filariæ*; he was a lazy man, slept much during the day, and in a very dark room such as mosquitoes affect even during the day, hence many of the insects he found in his net were charged with blood drawn at a time when the *Filaria* is not in the general circulation.

Coolie No. 3 brought on one occasion two, and on another occasion five mosquitoes. Their examination gave this result:—

Mosquito examined on 2nd day	No *Filaria*.
,, ,, on 3rd day	,,
,, ,, at 74 hours	,,
,, ,, at 96 hours	One sausage-shaped *Filaria*.
,, ,, ,, ...	Two *Filariæ*.
,, ,, ,, ...	Many *Filariæ*.
,, ,, ,, ...	Several *Filariæ*.

Again my theory seemed doomed to collapse. But I examined the man's finger-

blood in the evening with the same results as in coolie No. 2; it contained plenty of *Filariæ*.

Coolie No. 4 brought six mosquitoes with this result, that three of them when examined ninety-six hours after capture were found to contain *Filariæ*, and three of them yielded no parasitic form whatever. This man's blood was examined at 9 P.M., and, like that of coolies No. 2 and 3, was found to contain many *Filariæ*. These coolies all came from Hoorcoah, a district to the north of Amoy, in which a very large proportion of the inhabitants are filariated.

It was now evident I must examine mosquitoes from some less questionable feeding-ground before I ventured to draw any conclusion from this endeavour to prove a negative. I had a boy of about 15 years of age for a servant; he was healthy and his blood did not contain *Filariæ*. I got this lad to bring me his mosquitoes, with this result:—Eight mosquitoes examined sixty-four hours after capture—no *Filariæ* found; one mosquito examined eighty hours after capture—no *Filariæ*; one mosquito examined eighty-eight hours after capture—no *Filariæ*; five mosquitoes examined one hundred and four hours after capture—no *Filariæ*. I examined a few mosquitoes fed on other non-filarious individuals, and with the same negative results.

Many years ago, in searching for the intermediary host of the *Filaria immitis*, I examined many tens, if not hundreds, of mosquitoes that had fed on dogs' blood; but in no instance did I remark, although I was on the outlook for such, an advanced form of filarial development.

Lewis also did not find sausage-shaped nematodes in any mosquitoes but in those that came from a certain room where it was afterwards discovered that a filariated man slept. He examined with a negative result many mosquitoes that had preyed on the blood of dogs or birds.

Similarly Sonsino found *Filariæ* in about half his mosquitoes; they were captured in a bed where two men slept, one of whom had *Filariæ* in his blood, whereas the other was not thus infested.

The inference from these facts is obvious. It is only mosquitoes that have fed on the blood of filariated men that contain the developmental forms I have described.

In the foregoing I think I have conclusively shown that the mosquito fed on filariated human blood ingests the embryo *Filaria sanguinis hominis*, that while in the mosquito the *Filaria* undergoes great developmental changes, and that it finally quits this insect as a large and powerful animal equipped for an independent life. I have also shown that the mosquito fed on non-filariated blood exhibits, when dissected, no such parasitic forms. I therefore infer that the parasitic forms I meet with in the mosquito fed on filariated blood are really advanced forms of the embryo *Filaria* ingested with the blood, and that the mosquito is the proper intermediary host of the *Filaria*. I do not see how this inference can be avoided. It would be much more satisfactory, and an easier method of demonstration, could we watch an individual *Filaria* in its progress from the circulation down the proboscis of the mosquito into its stomach, watch it wriggling there, cast its skin, and, changing its method of movement, worm its way through the tissues into the thorax, become passive there among the muscles while it acquired size, an alimentary

canal and other organs, and enhanced muscular power; then eat or bore its way through the integuments of the dying mosquito, and finally obtain liberty in the water into which the insect has fallen. It is impossible to do this; but, by comparison of a large number of dissections, we can follow the history of the animal in the mosquito almost as perfectly as if we had watched in transparent tissues the progress of a single individual from the human body to the water.

The experimentum crucis of this theory, as I have already said, I have not had the hardihood to attempt. But from what I have written, any one anxious to make it will have no difficulty in gathering what is likely to be the most successful method. Were I to attempt it I would proceed in this fashion:—I would feed my mosquitoes on a filarious subject, I would collect them every morning, giving each a bottle to itself. Those that survived to the afternoon of the seventh day I would transfer to test-tubes; these I would invert over a watch-glass, containing a little water. When the insect died I would allow it to remain on the water a few hours and then remove it. The water it had fallen into, and which probably now contains the *Filaria*, I would transfer to a stock-bottle containing water. This process I would repeat for several days. I would then administer portions of the contents of the stock-bottle to the subject of the experiment. I would continue this for a month, every day adding fresh mosquito-water to the stock-bottle, and every day administering a draught of its contents. After a time I would commence the examination at night of the finger-blood. I am quite satisfied as to what would be the result.

EXPLANATION OF PLATE XXXIX.

[All the figures with the exception of figs. 10, 34, 35, 37, 43, 44, 45, and 46, are magnified about 188 diameters.]

Fig. 1. The embryo *Filaria sanguinis hominis* as it appears in the blood, or lymph, or in the abdomen of the mosquito immediately after ingestion, $\frac{1}{90}'' \times \frac{1}{3000}''$.

Fig. 2. A *Filaria* about an hour after ingestion by the mosquito. The sheath has been cast, transverse striation and oval pouting are very distinct, and the animal is indulging in the snake-like wriggling by which it moves from the abdomen to the thoracic viscera. In the mosquito from which this specimen was obtained many *Filariæ* were found in the newly ingested blood in the insect's abdomen. All were active and transversely striated. Most had cast the sheath. In one the sheath was lying at some distance, in another it trailed after the animal, while in a third it lay across it. Oral pouting was distinct in all; but no double outline, or further structure, could be detected in any of them. In the same insect two *Filariæ* were found in the thorax; they were structureless, without sheath, somewhat swollen from endosmosis, an obscure convoluted granular-looking condensation occupying most of the body.

Fig. 3. From the thorax twelve hours after ingestion, $\frac{1}{90}'' \times \frac{1}{3000}''$.

The abdomen of the insect was half filled with blood, in which moved many active, transversely striated, pouting *Filariæ*. They had no sheath. In the thorax many were found.

They showed no double outline, transverse striation, or sheath; but oval pouting and some general body-movement still persisted. As yet tail-differentiation was not discernible. Beyond a few undefined specks the body was homogeneous. Perhaps the mouth possessed four lips. Slide prepared with sulphate-of-soda solution gradually washed away with pure water.

Fig. 4. From the thorax 25 hours after feeding. The abdomen of the mosquito still contained fluid, or semifluid blood, about two thirds full. In this eight passive *Filariæ* were found; they were granular and evidently dead or dying. Over two dozen *Filariæ* were found in the thorax, most of them active, with differentiation of the tail commencing. That represented measured $\frac{1}{100}'' \times \frac{1}{2500}''$.

Figs. 5, 6. From the thorax 35 hours after ingestion. The abdomen of the insect was about two thirds filled with blood, in which 12 or 13 undefined, granular, dead, and fading *Filariæ* were found. One of them, however, which evidently had been about the periphery of the clot, was well defined, faintly striated, plump, moving freely, and had a tail slightly differentiated. Many were found in the thorax; all of these were passive and had distinctly differentiated caudal appendages. No organs seen; body-contents granular. The body appears to be expanding, the enlargements being most apparent towards the tail end. One measured $\frac{1}{100}'' \times \frac{1}{3000}''$, another $\frac{1}{140}'' \times \frac{1}{2500}''$. One or two exhibited slight movement, either slow bending and extension, or, at long intervals, sudden jerking extension.

Fig. 7. From the thorax, 39 hours. Large number of *Filariæ* in the thorax. For the most the tail was well differentiated. The body of the parasite had become thicker and shorter. In some, on washing away the sulphate of soda solution, a double outline could be made out. Contents cloudy and granular. Jerking movements frequent. A very few of the *Filariæ* were shaped and moved as in mosquitoes examined a short time after feeding; that is, they were long, slender, active, with a tapered tail. Between these and the distinctly tailed animal transition-forms were abundant, so that there could be no doubt about the connexion. Specimen represented measured $\frac{1}{125}'' \times \frac{1}{2500}''$.

Figs. 8–10. From the thorax of mosquito 46 hours after capture. Many *Filariæ* were found in this insect at different stages of development, from the long, slender, rather active *Filaria* with but slightly differentiated tail, to the fat tailed sausage-form with granular contents and intermittingly wagging tail. Fig. 10 shows the chitinous integument, dipping into the rudimentary mouth; it is drawn to a larger scale. In one field of the microscope three *Filariæ* lay across each other; one had hardly made any progress in development, another was still slender, but the tail was differentiated, while the third was thick, plump, and sausage-shaped.

Figs. 11, 12. From the thorax 56 hours after feeding.

The insect was found dead at 2 P.M., but was seen alive the same morning. Many live *Filariæ* found in this insect, exhibiting the extension of the body and flick of tail movement. Fig. 11 showed some body-extension and a remarkable vibration of the whole of the posterior part of the body. The contents were in marked contrast to those of No. 12, being a structureless homogeneous matrix interspersed with innumerable, well-defined, shining granules; it measured $\frac{1}{125}'' \times \frac{1}{1700}''$. Fig. 12 measured $\frac{1}{145}'' \times \frac{1}{1250}''$; it possessed no body-movement, but only the tail-flick. Its body was made up of distinct cells; one minute, glistening, nucleated, cell-like body was a prominent feature at a point where the anus might be. Many more *Filariæ* like these two types in the slide.

Figs. 13, 14. From the thorax 72 hours after capture. Many *Filariæ* much broader, and somewhat longer, than those from the mosquito of figs. 11, 12, which belonged to the same batch of insects, and examined the day before. The tail was not seen to move, and no movement of body could be detected. Obscure cellular arrangement of the body. In all specimens a crowd of granular matter escaped from the anus. Fig. 14 measured $\frac{1}{110}'' \times \frac{1}{700}''$ anteriorly, and $\frac{1}{625}''$

posteriorly; it was slightly constricted in the middle. After the slide had been mounted some time, a shrinking of the contents showed the integument of the parasites very distinctly, all exhibiting a marked double outline.

Fig. 15. From the thorax of a mosquito the date of whose feeding could not be fixed, probably about 80 hours before examination. In its thorax two *Filariæ* were found, one partly crushed, the other perfect—as represented. Measurements $\frac{1}{110}'' \times \frac{1}{1000}''$ anteriorly, and $\frac{1}{800}''$ posteriorly. It exhibited occasionally spasmodic jerks. At the head a distinct double outline. An alimentary line ran backwards some distance, becoming convoluted posteriorly, and gradually becoming less distinct till it was lost at the middle third. Anus distinct, open, and emitting a few granules. Tail distinct. Contents of the body obscurely cellular, lobulated, granular.

Fig. 16. From thorax $97\frac{1}{2}$ hours after capture. A considerable number of *Filariæ* found resembling sketch. Body smooth in outline, plump, and healthy-looking. The mouth was four-lipped, and a pharynx could be made out; but no other trace of an alimentary canal unless in one specimen in which a funnel-shaped anus was apparent in the usual situation. The tail was delicate, but moved distinctly. There seemed to be a condensation of tissue near the surface of the body, especially about the waist. There was a shoulder and an anal bulging. Movement of the lips was observed. Measurements $\frac{1}{120}'' \times \frac{1}{1200}''$.

Fig. 17. From thorax 97 hours after capture; about a dozen sausage-shaped *Filariæ* found. Four lips readily made out; alimentary line running from mouth to anus. Cellular matter escaping from anus. Contents of body cellulo-granular, and very obscure. Tail small. Specimen measured $\frac{1}{110}'' \times \frac{1}{750}''$.

Figs. 18, 19. From the thorax of a *Filaria* found dead (but recently living) 96 hours after capture. Alimentary line very distinct. Body cellulo-granular. Four lips rather pursed up. One measured $\frac{1}{110}'' \times \frac{1}{715}''$. Many similar in this insect.

Fig. 20. From thorax 144 hours after capture. In this particular insect eight such *Filariæ* were found. Mouth, alimentary canal, and anus, well marked in all. Tail was very faint, and it could easily be ascertained that at this stage it was nearly entirely integumented. The longest measured $\frac{1}{80}'' \times \frac{1}{650}''$. In it the cellular structure of the body was very apparent posteriorly; towards the head the cell-outlines could not be made out, the tissues looking granular or homogeneous. The mouth was pursed up. No movement remarked; granular escape from anus considerable. Alimentary line very thick, giving the impression that it was filled with some dark substance.

Figs. 21–23. From the thorax of a mosquito 128 hours after capture. In this insect about a dozen advanced *Filariæ* were found. They were nearly all straight, extended, with truncated posterior ends tipped with a minute, delicate, tegumental tail. Mouth distinctly four-lipped. Shoulder and anal bulging, and waist, inmost. Alimentary canal traceable from the mouth to anus in some, in others gradually losing itself about the middle of the body. Round this line nucleated cells have ranged themselves. In fig. 21 a prolapsus of part of the intestine has occurred. The body is filled with minute, clear, nucleated cells, in all the specimens. In 23, in addition to the alimentary line, traces of what may be commencing organs of generation were seen; a line could be followed from a point a little posterior to the head meandering backwards for some little distance among the cells. Where this line opened on the surface a dimly indicated infundibulum, which may be vulva and vagina, was indicated. Outline of all distinctly double. Fig. 23 measured $\frac{1}{85}'' \times \frac{1}{500}''$; the others about $\frac{1}{90}'' \times \frac{1}{850}''$ and $\frac{1}{85}'' \times \frac{1}{650}''$.

Fig. 24. From thorax 145 hours after capture. Eight *Filariæ* in thorax far advanced in development. The mouth and tail very evident. In some the tail was entirely integumental; in others a papilla from the body projected slightly into it—fig. 34; whilst in others the integument was quite filled up with the same material that made up the bulk of the body—fig. 35. When first

seen, *i.e.* soon after the body of the insect was immersed and broken up in water, part of the alimentary canal was visible as a distinct tube, and the body was for the most part very clear. After a time the alimentary canal became obscured by the cellules becoming granular and confused. Granular matter escaped from the anus. The cellules were smaller than those in figs. 21, 22, & 23. Several parasites were crushed under the cover-glass; in these chitinous integument was readily seen. Measurement $\frac{1}{75}'' \times \frac{1}{625}''$.

Fig. 25. From the thorax of a mosquito found dead on the morning of the seventh day after capture. The arrangement of cells round the alimentary canal, and on the inside of integument, very easily made out. The cells were plump and nucleated. In addition to the peripheral layer of cells the walls of the body appeared to be strengthened by a sort of thick corium, to the inside of which the cells were attached. There was a distinct space, a sort of peritoneal cavity, between the cortical and intestinal cells. In one specimen the head moved slowly at intervals. Mouth in all pursed up.

Fig. 26. From thorax. Mosquito found dead 144 hours after capture, seen alive 8 hours before. Eight such *Filariæ* found. Two of them had slight movement; the largest measured $\frac{1}{60}'' \times \frac{1}{715}''$; another $\frac{1}{65}'' \times \frac{1}{715}$. The sickle-shaped tail was purely integumental, very faint and fine. The body in some projected into this as a papilla; in others it was abruptly truncated. In most the anus was a large bag with well-defined wall, and from it granules escaped. Alimentary canal traced as a tube two thirds down the body, but union with rectum not distinctly discerned. Mouth widely open. Cellular structure of body obscure. Many shining granules in the wall of the alimentary canal.

Fig. 27. From thorax of mosquito found dead and adhering to damp side of bottle, 132 hours after capture. Altogether there were about six *Filariæ* in the insect's thorax, and all in an advanced state of development; mouth, anus, alimentary canal, distinct; body cellulo-granular. Mouth in every case pursed up so that the lips were with difficulty defined. The specimen represented was more advanced than the others. The anterior part of body had thinned down and lengthened, while the posterior part remained full size, giving the animal the hock-bottle appearance. The cephalic end very transparent, alimentary tube in it barely visible. Mouth pursed up, and no lips discernible. Cephalic end exhibited slow swaying movement when fresh. Measurements $\frac{1}{85}'' \times \frac{1}{1250}''$ anteriorly, and $\frac{1}{625}''$ posteriorly.

Fig. 28. From mosquito 135 hours after capture. Many advanced *Filariæ* found in this insect. Some of them showed thinning and commencing extension of cephalic end. The specimen represented had grown at both extremities, particularly at the cephalic; but a portion of the middle of the animal had not yet become extended, hence its peculiar appearance. The entire animal measured $\frac{1}{55}''$ in length; the neck was $\frac{1}{1000}''$, the posterior part $\frac{1}{650}''$, and the bulging centre $\frac{1}{500}''$ in diameter. Lips distinct; body cellulo-granular; tail, a long delicate sickle. Some motion exhibited.

Fig. 29. From a mosquito 144 hours after capture. Many advanced *Filariæ* in thorax, some of them undergoing stretching. One measured $\frac{1}{50}'' \times \frac{1}{625}''$; another $\frac{1}{60} \times \frac{1}{710}''$. Œsophagus could be made out as a thick-walled tube in most; in some it terminated in a bulbous valvular-looking arrangement, from which sprang the intestine. Mouth distinctly lipped; a pharynx seen in some. In some no tail could be detected, in others a very transparent sickle was seen. Body cellulo-granular; no movements.

Fig. 30. From mosquito 152 hours after capture. About 15 large *Filariæ* were found in the thorax. The largest is represented, it measured $\frac{1}{40}''$ by $\frac{1}{615}''$ posteriorly, $\frac{1}{700}''$ anteriorly. A number were found crowded together in one corner of the slide at various stages of extension from the $\frac{1}{100}''$ up to the $\frac{1}{50}''$ in length. Cellular structure of the body in some most apparent. Œsophagus in some particularly distinct, the single layer of nucleated cells constituting the wall

showing out well. Mouth open, four-lipped, with signs of papillæ on skin (fig. 37). In some no trace of tail, in others a large beautifully transparent sickle into which the body dipped a stumpy papilla. Rectum very evident. Granules in some escaping. A little, but very little, movement in some heads. No trace discoverable of organs of generation.

Fig. 31. From the thorax of a mosquito 156½ hours after capture. The ova had been deposited. In this insect *Filariæ* were found at almost all stages of development, from large "sausages" to almost the *Filaria* of the sixth stage. One of the latter measured $\frac{1}{20}'' \times \frac{1}{1000}''$, and had three distinct papillæ on the tail. In this instance motion was most active in the tail, but the head also was animated; a prolapsus of intestine took place about the middle of the body. In another, about $\frac{1}{25}''$ long, the alimentary canal was very distinct, but rapidly became granular; hernia occurred in two places, and the tail and head retreated from their integuments; in the case of the head it left unoccupied about $\frac{1}{1000}''$ of the integument, and in this the involution to line the pharynx was very distinct. In some specimens the transparent sickle-shaped tail still remained.

Figs. 32, 33. The former is somewhat diagrammatic; the latter was constructed from observation of two full-grown *Filariæ* at the sixth stage. The following are my notes of examination of the particular insects in which the originals were found:—

(*a*) A large brown mosquito, 158½ hours after capture, with ova still undeposited. Many *Filariæ* were found in the thorax, in the fifth stage, measuring from $\frac{1}{30}''$ to $\frac{1}{40}''$, or thereabouts. One *Filaria* had attained a more advanced stage; it measured $\frac{1}{16}'' \times \frac{1}{825}''$. It possessed great activity, for it was in incessant motion, wriggling, coiling, and uncoiling. One movement was peculiar; it slowly, slightly approximated one end of the body to the other, and then suddenly extended itself. The motion was too quick for the eye to follow. The viscera were very difficult to make out. There were papillæ at one end of the body, the other end was tapered, and then abruptly rounded off. No trace of organs of generation visible. No trace of alimentary canal, or anus, could be made out, all details being obscured either by extreme opacity or extreme transparency of the body. Unlike *Filariæ* at an earlier stage, this individual seemed quite at home in the water; the longer it remained in it the more active it became, and there was no bulging of the body or escape of contents. The other *Filariæ* found in this mosquito, after moving languidly for a time, gradually became less active, and finally passive. Their bodies at one or two places would bulge and then rupture, intestine protruding. In these *Filariæ* details of structure were easily made out. The large *Filaria* was visible to the naked eye.

(*b*) A large brown mosquito, 159½ hours after capture, eggs still undeposited. The thorax contained about 8 *Filariæ*, "sausages" for the most part at an advanced stage. One measured $\frac{1}{50}''$; another measured $\frac{1}{16}'' \times \frac{1}{825}''$. The fore part of the body of this specimen was in constant, active, swaying motion; and the posterior half of the body apparently in the advanced fifth stage, *i. e.* it looked more cellular, was affected by endosmosis, and possessed little motion. The fore part had a firm, fibrous look, and the alimentary canal could sometimes be seen moving inside the muscular and fibrous walls, accommodating itself to every movement. The mouth was firmly pursed up, and the outline of the lips was quite indistinguishable. As it turned at times towards the eye, the very centre seemed as if pointed to a spine, a ring of four or more little spines, or papillæ, surrounding it; this is to some extent imagination, the perpetual movement preventing reliable observation. On the extremity of the tail were two, perhaps three, large, rounded papillæ; close to these, about two diameters of the body in advance, the anus and rectum could be distinctly made out. A hernia of the intestine occurred after this animal had been under observation about an hour. The hernia consisted entirely of intestine, no trace of uterine tubes or testicle. The movement of the head end continued for an hour and a half,

apparently brisker at the end of this time than at the beginning; it was only suspended when the cover-glass was removed, and the slide dried.

Figs. 34, 35. Show with fig. 24 the process of withdrawal of the body from the tail.

Fig. 36. Probably a dead *Filaria* with irregular bulgings, vacuoles, and signs of degeneration.

Fig. 37. Head of fig. 29 more highly magnified, and drawn at a slightly later hour, when the body on dying had somewhat retreated from the integument.

Figs. 38 & 39. From the same mosquito, showing differences in the rate of progression of metamorphosis.

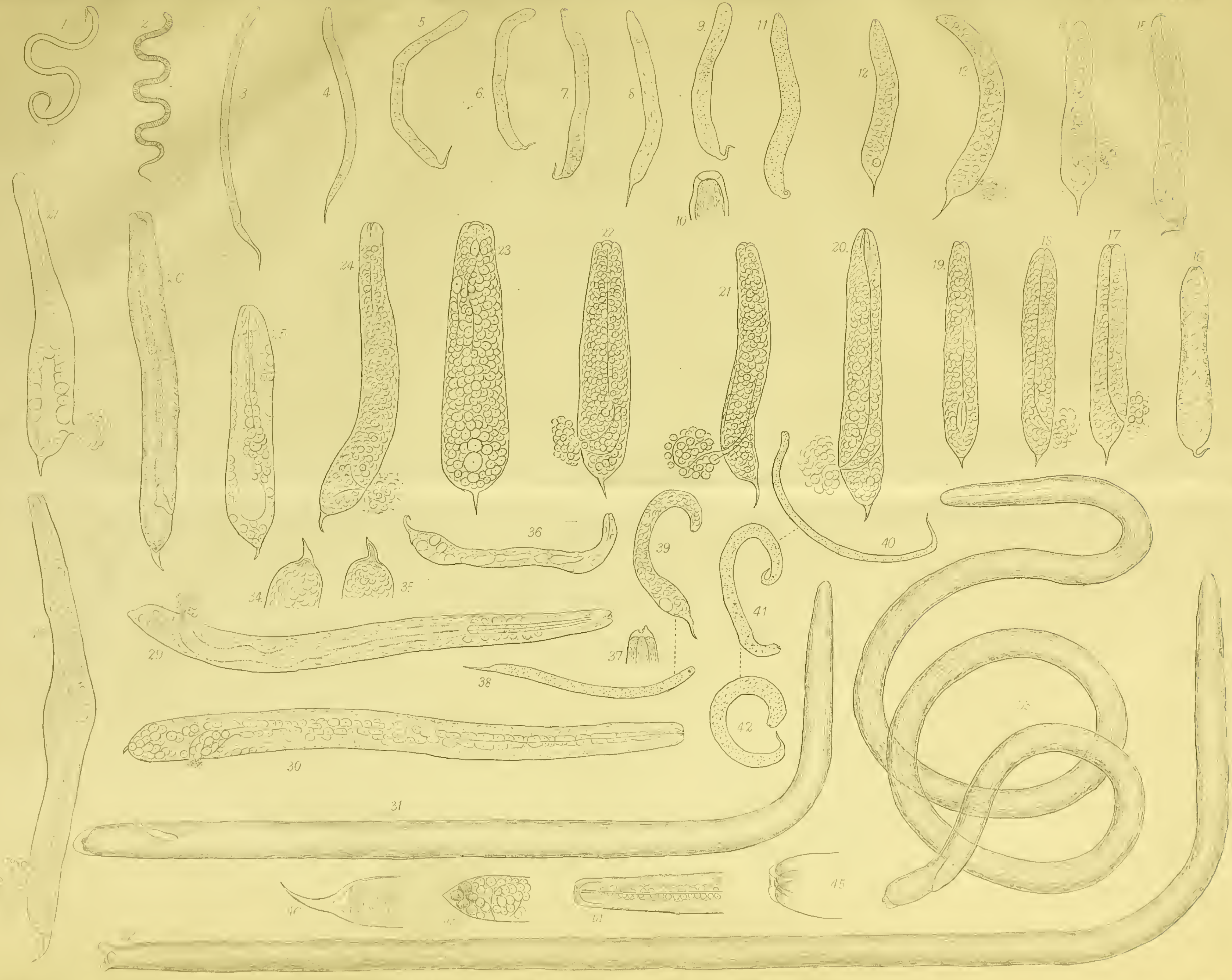
Figs. 40, 41 & 42. Also all from the same insect.

Fig. 43. Caudal papillæ developing.

Fig. 44. Pharyngeal lines very distinct, from a *Filaria* in the fifth stage.

Fig. 45. Showing a sort of horny thickening of the tips of the lips at the fourth and early fifth stages.

Fig. 46. The sickle-shaped integumental caudal appendage not yet shed; caudal papillæ developing, and possessing a delicate skin separating them from the original integument of the earlier stages.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